STRUCTURE OF THE YEAST CELL ENVELOPE

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STRUCTURE OF THE YEAST CELL ENVELOPE

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ABSTRACT. The structures of cell walls and plasma membranes are studied, by the freeze-etching method. A diagram of the cell envelope proposed by the author is presented.

1. Introduction

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The yeast cell envelope can be largely classified into a cell wall and a plasma membrane. Rose (1963) [1] claims that the cell wall thickness is 1/7 of the cell diameter. Also, according to Narthcote (1954 [2], the cell wall comprises 15% of the whole cell, although Falcone and Nikerson (1956 [3] think it is 30%. At any rate, the yeast cell envelope occupies the greater part of the cell. As regards the cell structure, Conway and Downey (1950) [4] advocate a two-layer theory. The outer layer is a thick membrane cell wall which permits the passage of macro molecules, while the other layer is the inner layer which is permeable to ions. Concomitant with the development of electron microscopy technology, detailed studies have been made of the structure of the outer layer. Northcote, Horne (1952) [5], Mundkur (1960) [6], and Rose [1] support the two-layer theory, while Bartholomew, Levin (1955) [7], Agar, Douglas (1957) [8], and Marquardt (1962) [9] are in favor of the three-layer theory. Vitols (1961) [10] observed a mono-layer in the electron microscope image that had been fixed with potassium permanganate. Concurrent with research on the cell wall structure, studies have been made on the plasma membranes. Robinow and Murry (1953) [11] introduced a technique that will

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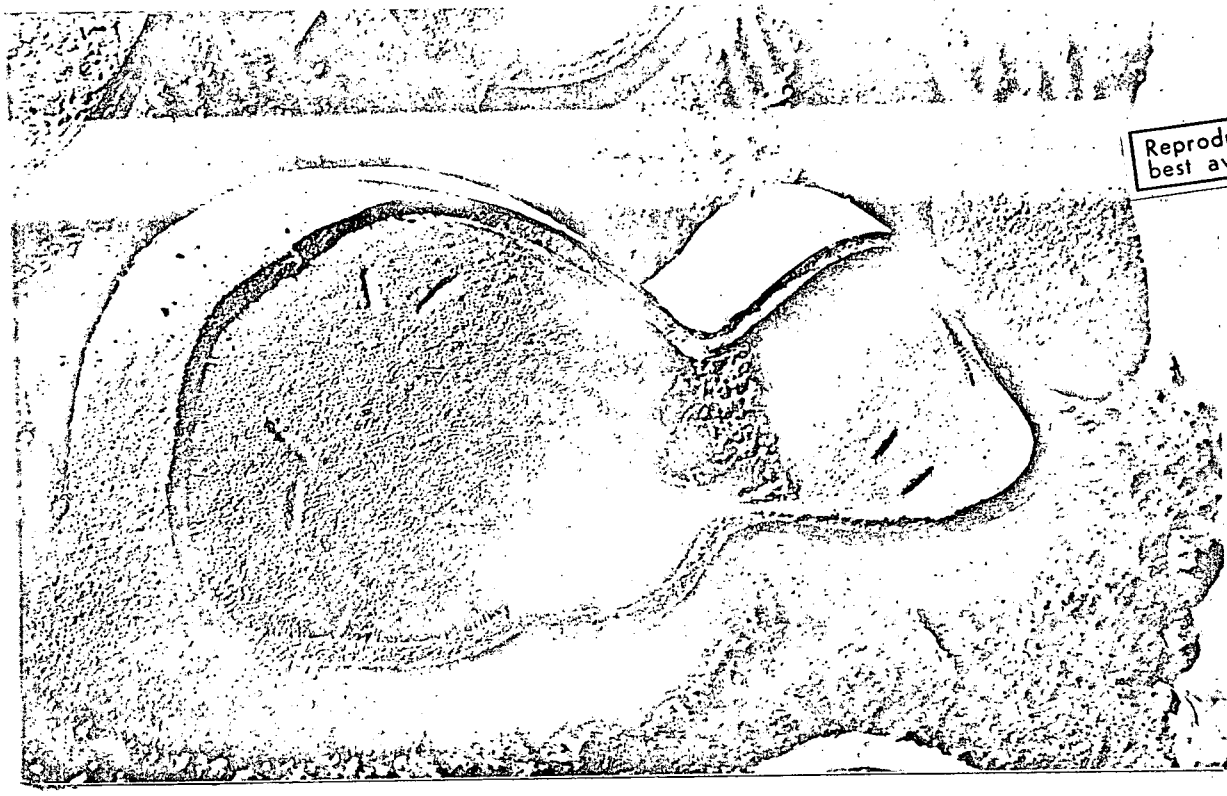
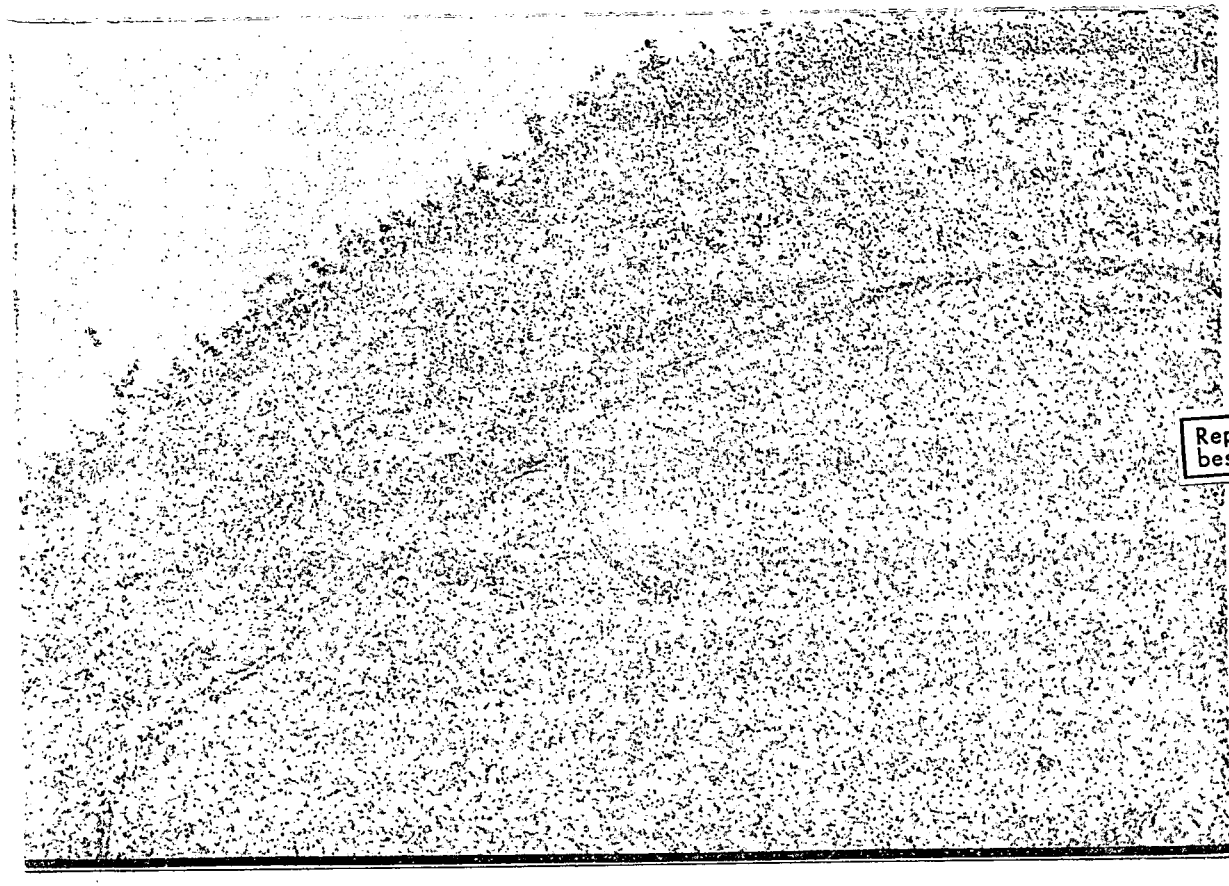
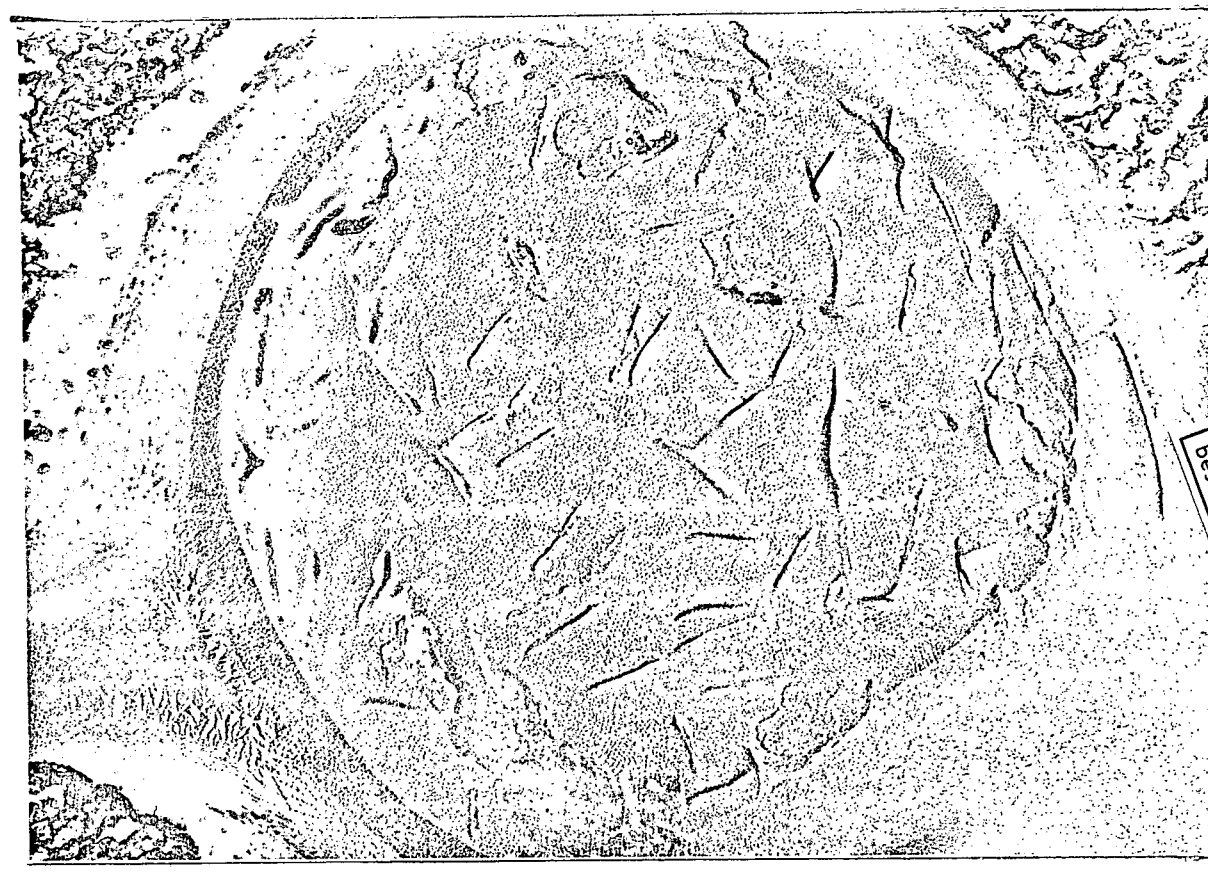


Photograph 2. Structure of the plasma membrane (Torulopsis glabrata) of germination cells. Left: surface of the plasma membrane of a mother cell. Several invaginations are visible. Right: inside of the plasma membrane of a daughter cell. Three invaginations are visible (by Dr. Edwards) X 20,000.



Photograph 3. Structure of the plasma membrane and the cross section of the cell wall of Saccharomyces fixed with $KMnO_4$. Invagination of plasma membrane and unit membranes are shown. X 300,000.

Photograph 4. Structure of the plasma membrane surface of bread yeast (Saccharomyces cerevisae) by the freeze-etching method. Invaginations and granules are visible. X 20,000.



Photograph 5. Structure of the plasma membrane surface of Schizosaccharomyces pombe by the freeze-etching method. Invaginations are visible, but granules do not exist (by Okuma and Washio) X 30,000.

selectively dye cell walls and plasma membranes using victorial blue-B. Development of the electron microscope technique clarified the existence of plasma membranes, and also revealed that they have a unit membrane structure. The latter finding was ascertained by the freeze-etching technique.

In this article, entitled "Structure of the Yeast Cell Envelope," the structures of cell walls and plasma membranes will be studied.

2. Cell Wall

a. Composition of Cell Wall

Regarding the chemical composition of cell walls, Northcote (1954) [2], Northcote and Horne [5] carried out the analysis on a detached cell wall. According to the analysis, it is composed of 29% glucan, 31% mannan, 13% protein, 8.5% fat and 3% ash. As a result of the electron microscope analysis, it is composed of at least two layers: one is a glucan layer, and the other is a mannan-protein complex.

Eddy and Rodin (1957) [12] used many varieties of yeast cells and observed the cell wall structure by the enzyme treatment technique. As a result, they confirmed the presence of a partial mannan layer in the outer layer of cell walls.

Using an optical microscope and an electron microscope, Mundkur [6] took freeze treated yeast and confirmed the presence of the localized polysaccharides in the cell walls by the PAS reaction.

As a result of the alkaline and enzyme treatments, the natural state of cell walls is assumed to be a complex polymer.

Phaff (1963) [13], Nickerson (1963) [14], and Northcote (1963) [15], confirmed that cell walls are composed of glucan (yeast cellulose), Mannan (yeast gum), chitin, protein, fat, and ash. Using an electron microscope,

Houwink and Kreger (1953) [16] reported that glucan is the only polysaccharide, and that the hydrolyzed glucan is fibrous. Northcote (1963) [15] reported that the amount of glucan in Saccharomyces cerevisiae is 30% of the dried cell wall. Northcote also reported that the amount of the other important component (i.e., mannan) in Saccharomyces cerevisiae is the same as in glucan. Kregar (1954) [16], however, reported that there is no mannan in Schizosaccharomyces, Nodsonia, and Rhodotorula. /16

Chitin remains as an insoluble residue after mannan, glucan, protein, and fat are completely removed. The presence of this substance was discovered by Houwink and Kreger (1953) [17] by x-ray diffraction. Although there is 2 - 3% glucosamine in cell walls, less than 10% of glucosamine changes to chitin. Roelofsen and Hoette (1951) [18] think there is no chitin in Schizosaccharomyces octosporus.

Protein is an important component of the yeast cell wall. Falcome and Nikerson (1956) [3] report that it comprises 7% of the dried weight. The amount, however, depends on the culture conditions, time, and the variety of the yeast. Although the cell wall protein is comprised of a great many amino acids, Kessler and Nickerson (1959) [19] confirmed that it is especially abundant in glutamic acid and aspartic acid.

Nicherson (1963) [14] reports that the amount of fat varies from less than 1% to more than 10% depending on the variety. The cell composition has been analyzed, and is known chemically and structurally. Lampen (1968) [20] showed the structure of a Saccharomyces cell wall (Figure 1). /17

b. Cell Wall Configuration

With the development of electron microscope techniques, the cell wall structure was clarified. Marquardt (1962) [9] reported on the three-layer cell wall structure by fixing it with potassium permanganate. According to his theory, there are two high electron density layers inside and outside the

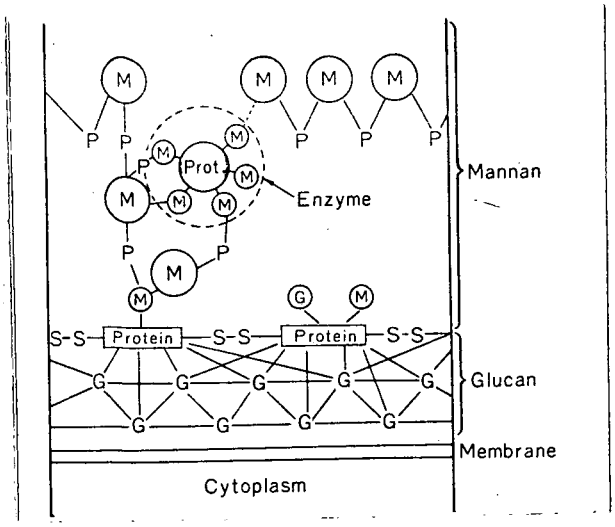


Figure 1. Structural diagram of the yeast cell wall. M - mannan, G - glucan, P - phosphoric acid, S - sulphur (from Lampen 1968).

cell wall with a low electron density layer in between. This is confirmed by the observation made with the freeze-etching technique. The outermost layer is composed of mannan-protein that is stained by potassium permanganate fixation. Chemical studies on cells (Mundkur (1960) [6]) on a frozen sample revealed that the layer contains chittin. Mundkur also found that the central unstained part is a glucan layer. Houwink and Kreger (1953) [17] observed with an electron microscope that the acid-treated cell wall assumes a fine fibrous structure. Streiblová (1968) [21] observed by the freeze-etching technique that this is the natural arrangement of glucan molecules (Figure 2). She observed the protoplast formation by the snail enzyme treatment. In this case, the innermost layer of the cell wall — that is, the outer layer of the plasma membrane — was not completely detached from the plasma membrane. Although Hirano et al. (1968) [22] formed protoplasts with a snail enzyme and observed the plasma membrane structure in an ultra thin slice, the residue of the innermost layer of the cell wall in the membrane structure was not observed.

c. Regeneration of Cell Wall

The regeneration of cell walls of enzyme protoplasts has long been discussed. Necas (1956 [23], 1961 [24], 1965 [25]) reported on the new generation by germination. As previously mentioned, Streivlova claims that the residue of the innermost layer of a cell wall, subsequent to the protoplasts formation, serves as a nucleus for the regeneration of cell walls.

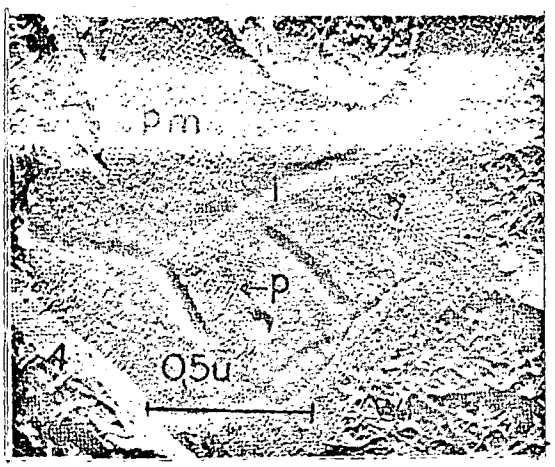


Figure 2. Photograph of enzyme treated Saccharomyces cerevisiae (by the freeze-etching technique). pm - plasma membrane, p - granule, i - invagination, wf - fibrous structure. (by Dr. Streiblová, 1968)

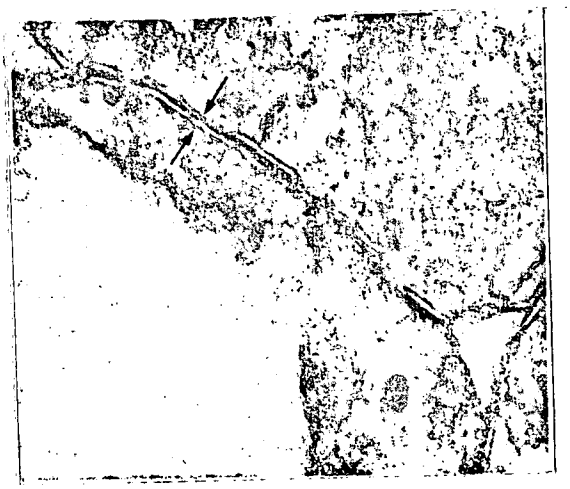


Figure 3. Plasma membrane structure of the yeast protoplasts. Arrows indicate th unit membrane structure of the plasma membrane. (Hirano et al., 1968)

d. Abnormal Formation of Cell Walls

Smith and Marchant (1969 [26] observed the growth of cell walls from 0.091 μ to 0.159 μ for a Rhodotorula glutinis cultured in chloramphenicol (500 μg/ml). It is believed that in the chloramphenicol there is an inhibitor to the synthesis of proteins which acts to remove the ingredients necessary for the formation of cell walls (Figure 4).

Such an abnormal cell wall synthesis, resulting from agents that inhibit the synthesis of proteins, has been observed.

e. Germination and Cell Wall Formation

Bud scar and birth scar are the names given to differentiate between a daughter cell and a mother cell. Barton (1950) [27] reports that normally birth scars are larger than bud scars (Figures 5, 6, 7). Nickerson (1963)

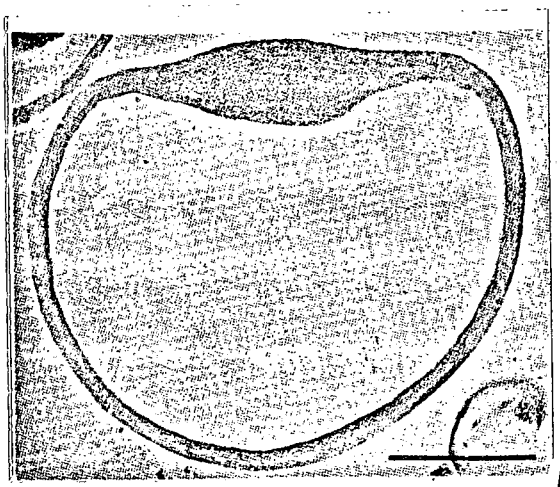


Figure 4. The cell wall of yeast cultured by the chloramphenicol addition. The thickened wall is shown (Drs. Smith and Marchant, 1969).

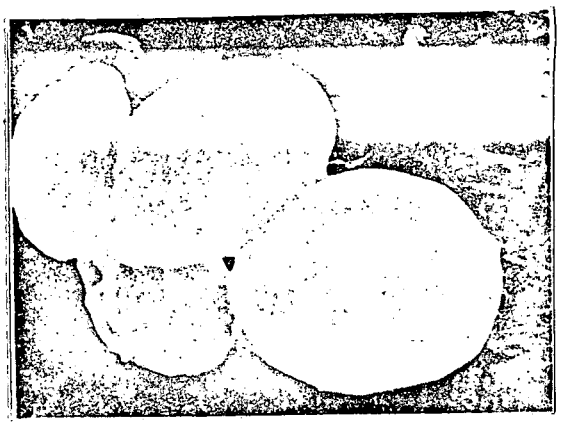


Figure 5. Yeast cells observed by a scanning electron microscope. Scan was made subsequent to the freeze-dry treatment; birth scars are visible. X 1,200.

Figure 6. Surface structure of a plasma membrane by the freeze-etching technique. Protrusion is visible in the germinating part (submitted by Dr. Edwards). X 15,000

[14] and Moor (1967) [28] believe /18
that, in order to produce germination, there should be a concentration of proper enzymes at the part, and a local weakening in the cell wall. Bud scars contain considerable amounts of chitin (Bacon et al. [29] 1960) and mannan-protein (Mundkur 1960 [6]). Merchant and Smith (1967 [30]) report that glucan fibers are contained in the wall separating the daughter and mother cells when new cell walls are formed by germination. In the separation of walls, the separation occurs from the center in the case of Saccharomyces (Figures 8, 9), whereas it occurs from the outside for Schizosaccharomyces pombe.

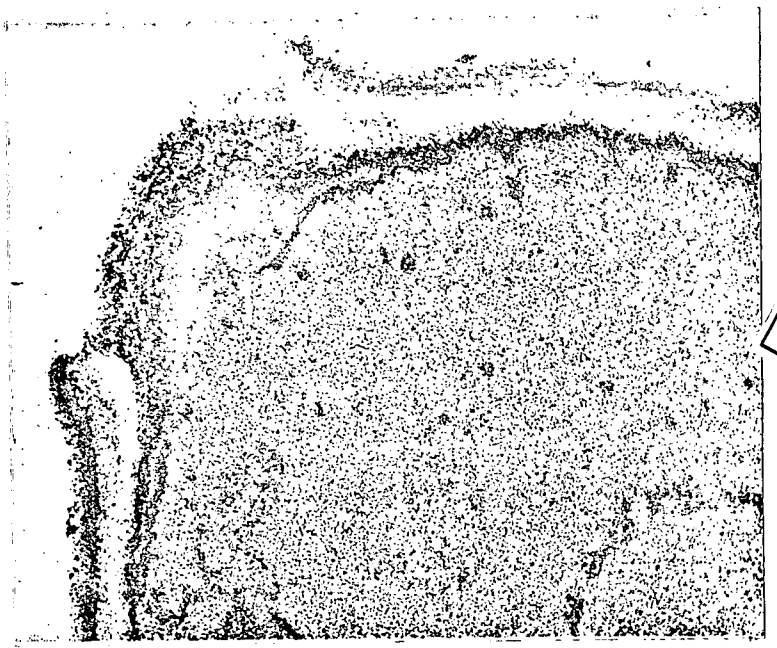


Figure 7. Bud scars in an ultra thin specimen. X 74,000.

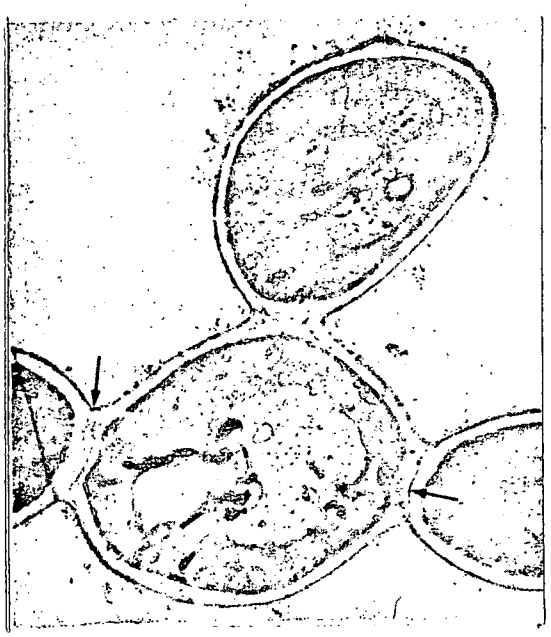


Figure 8. Germination cells of Sacchromyces cerevisiae. Detachment from the wall center can be observed. X 18,000.

3. Plasma Membrane

a. Configuration

As for the presence of plasma membranes, there are reports on attempts to observe it with an optical microscope by making use of the separation of plasma membranes in an alkaline or a salt solution. However, as mentioned previously, the plasma membrane structure of yeasts was probably first observed by Robinow [11] employing the staining technique.

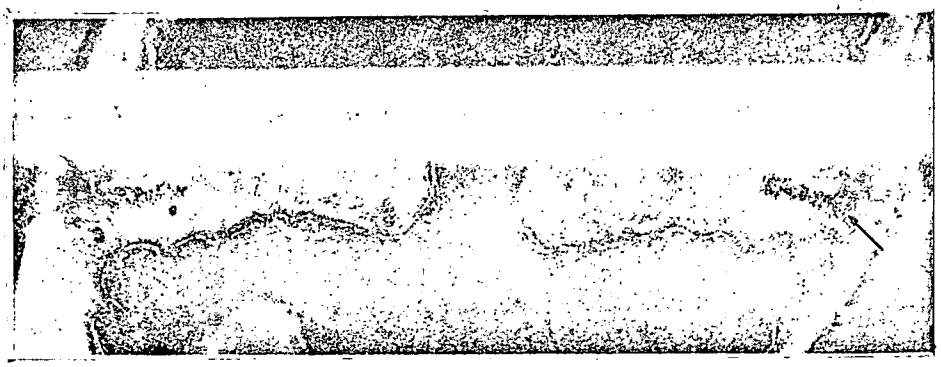


Figure 9. Division of Schizosaccharomyces pombe. Formation of walls and separation from both ends are visible (arrow). X 64,000.

Bartholomew and Mittwer (1952) [31] first observed plasma membranes by an electron microscope. They observed the plasma membrane of a cell dissolved by ultraviolet radiation. Later, the attempt by Bartholomew and Levin (1955) [32] with an osmium fixed ultra-thin slice resulted in failure.

Hashimoto, Conti and Naylor (1959) [33] reported on the presence of a plasma membrane with a specimen fixed with potassium permanganate.

Agar and Douglas [8], who established a remarkable record in the micro-structure study of yeasts, reported that, according to the studies made on osmium-fixed specimens, the plasma membrane structure has a highly invaginated configuration. At the time, however, it was suspected that this was an artificial product of the fixation process. Koehler (1962) [34], and Hirano (1961) [35] observed the same structure by potassium permanganate fixation. Hashimoto et al. (1959) [33] observed a smooth plasma membrane structure and asserted that the structure changed with growth. At the time, this was a /20
major problem concerning the micro-structure of yeasts. Later, the unit membrane structure, which was advocated by Robertson (1959) [36], was brought into yeast cells. Vitols [10] reported by the potassium permanganate fixation method that a plasma membrane is composed of 80 Å unit membranes. Later,

Hirano (1968) [22] observed the unit membrane structure with an ultra-thin slice of protoplasts' plasma membrane and supported the idea of Vitols. Robinow and Marak [37] reported on the existence of a plasma membrane in an osmium-fixed slice that had been pre-treated with snail enzyme. Although potassium permanganate fixation is a well-known and excellent method for the observation of membrane structures, glutaric aldehyde and osmium or potassium permanganate are reported to result in beautiful photographs.

b. Invagination of Plasma Membrane

It was mentioned earlier that, ever since Agar and Douglas first discovered invaginations in the plasma membrane structure, this discovery has stimulated many discussions. By the freeze-etching method, Moor (1964) [38], Moor and Mühlethaler (1963) [39] confirmed that such a structure is normal for the yeast plasma membrane. The size of the invagination is different for each cell; some are short, and others have many branches (Figures 10 a, b and Figure 11). The change in the shape of the invagination with acridine-orange* treatment has also been observed. The number of invaginations may depend on the age of the cell.

c. Surface Structure of Plasma Membrane

It has become possible to closely observe the surface structure of a plasma membrane by the freeze-etching method. The slicing method limited the observations to only the cross-section of membranes, and no method was available to determine the surface structure.

The freeze-etching method makes it possible to observe the front and back structures of membranes, the inner structure of cell walls, and the cross section of membranes. Aside from the invaginations found on the surface of /21

*Translator's Note: This is tetramethyl acridine.

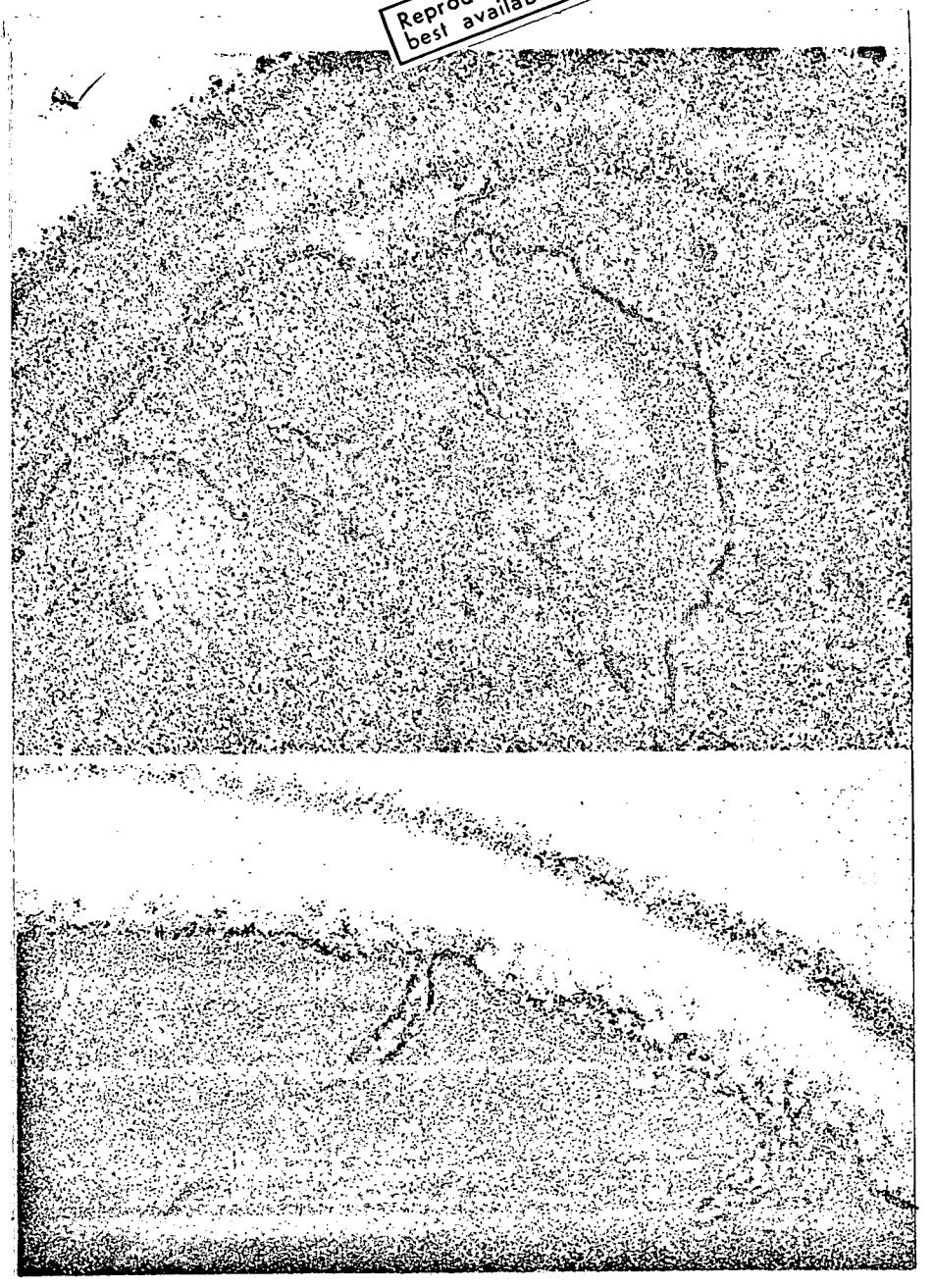


Figure 10. Invagination of a plasma membrane (ultra-thin specimen).

a (above): potassium permanganate fixation. X 185,000.
b (below): glutaric aldehyde. Osmium fixation. X 179,000.

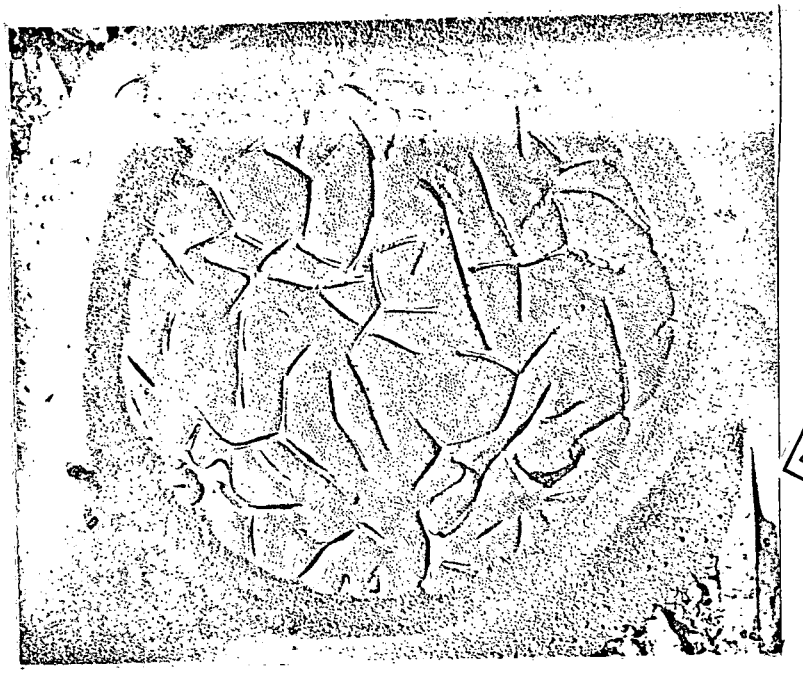


Figure 11. Surface structure of plasma membrane by freeze-etching method. Branched invaginations and numerous granules are seen. X 28,000.

plasma membranes, the criss-cross arrangement of approximately 15 μm diameter granules can be observed. Edwards (1969 [40] published a spectacular photograph showing cell walls and the structure of the front and back surfaces of a plasma membrane (cf photo 2). Granules on the front surface are considered to be the part where glucan fibers are synthesized. Although it is questionable whether or not these granules are artificially introduced by the freeze-etching technique, the fact that they are exclusively observed in the Saccharomyces family and not in Schizosaccharomyces makes it likely that it is a normal structure which merely depends on the cell wall composition (cf. Figure 11 and Photo 4).

d. Chemical Composition of a Plasma Membrane

From the analysis of plasma membranes it is known to consist of fat (including phospholipids), protein, and polysaccharide. The polysaccharide

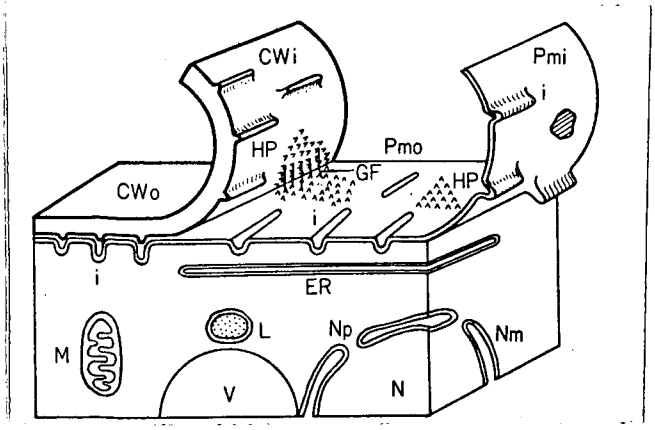


Figure 12. Diagram of yeast cell envelope. CWo - cell wall surface; CWi - inside of cell wall; Pmo - plasma membrane surface; Pmi - inside of plasma membrane (the cell side); HP - granules; GF - glucan fibril; i - invagination; ER - endoplasmic reticulum; V - vacuole, M - mitochondria; L - lipid granule; N - nucleus; Np - nucleus pore; Nm - nucleus membrane.

is known to hydrolyze only into mannose. The mannan is present with protein in plasma membranes. Also, it is characteristic that an ATPase-separated plasma membrane inhibits activation only in the presence of Mg ions. Matil (1967) [41] et al. report that it is different from the Oligomycin-proof mitochondria aTPase. Studies to determine the relationships between the structure and the function will probably expand in the future.

The structure of the yeast cell envelope (cell wall and plasma membrane) has been studied. A diagram of the cell envelope proposed by the author is shown in Figure 12.

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